

# Experimental Studies on Acute Gastric Mucosal Lesion Involved with Obstructive Jaundice : Mainly on the Changes of Amine Contents in the Gastric Mucosa on Cold Restraint Stress

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## ABSTRACT

The authors gave load of cold restraint stress to rats with obstructive jaundice and those on which biliary drainage was performed in order to examine the incidence of AGML, amine contents in the gastric mucosa, and gastric mucosal microcirculation, and the following results were obtained:

- 1) The incidence of AGML in the 3,4-week group of rats with obstructive jaundice and in the 4-week group of rats with biliary drainage after cold restraint for 30 min was increased markedly by 78% for each as compared with 22% in the control group.
- 2) The contents of histamine and serotonin in the gastric mucosa in the control group showed no significant change after cold restraint for 30 min, whereas the contents of both amines in the 3,4-week group of rats with obstructive jaundice and in the 4-week group of rats with biliary drainage were, respectively, significantly decreased after cold restraint.
- 3) Gastric mucosal microcirculation in the control group was prevented rather successfully, whereas that in the 4-week group of rats with obstructive jaundice after cold restraint for 30 min developed disturbance of gastric mucosal microcirculation due to congestion.

The authors paid attention to the amines existing in abundance in the gastric mucosa in rats, and the relationship of them with the incidence of AGML in obstructive jaundice. As a result it was suggested that there might be a possibility of the association of the increase in the storage of histamine and serotonin in the gastric mucosa with the formation of a preparatory state for AGML<sup>7)</sup>.

During the experiment, cold restraint stress was loaded on rats with obstructive jaundice and those on which biliary drainage was given, and experimental discussion was made.

## MATERIALS AND METHODS

### 1) Animal procedures

Wistar strain male rats (200 — 250 g) were classified into the non-treated control group, the 2-4 week of the obstructive jaundice group (the obstruction group), and the drainage group on which biliary drainage was given at each period of 2 to 4 weeks for 5 days<sup>1)</sup>, and they were fasted for 24 hr before using them for the experiment.

### 2) The method for loading of cold restraint stress

Cold restraint stress was loaded on rats for 30 min in accordance with Senay's method<sup>4)</sup>.

### 3) Determination of histamine (HA) and serotonin (5-HT) contents in the gastric mucosa

The fundic mucosa and antrum tissue were taken and homogenized according to the method of Wada et al<sup>8)</sup>, after which HA and 5-HT were extracted. Fluorescent quantitation of HA was performed by the method of Shore et al<sup>5)</sup> and 5-HT by the method of Maickel-Miller<sup>2)</sup>.

### 4) Method for the observation of the microvascular structure in the gastric mucosa

The animals were examined with the FITC-dextran method<sup>3)</sup> under Nembutal anesthesia (25 mg/g, i.p.).

### 5) Method for the observation of the distribution of erythrocytes within capillary systems in the gastric mucosa

Paraffin blocks prepared for the observation of the microvascular structure in the gastric mucosa were given H-E staining, and the distribution of erythrocytes within capillary systems in the gastric mucosa was observed.

Student's t-test was employed to determine statistical significance.

## RESULTS

### 1) Changes in the incidence of AGML

The incidence of AGML in the 3,4-week of the obstruction group and in the 4-week of the drainage group after stress was increased markedly by 78% for each as compared with 22% in the control group (Table).

**Table** Incidence of AGML on cold restraint for 30 min

Day after drainage	0	5th
Group		
Control	2/9 (22%)	
Obstruction 2W	3/9 (33%)	2/9 (22%)
Obstruction 3W	7/9 (78%)	3/9 (33%)
Obstruction 4W	7/9 (78%)	7/9 (78%)

### 2) Changes in the HA contents in the gastric mucosa

The HA contents in the control group were  $22.7 \pm 1.5 \mu\text{g/g}$  in the pre-stress level, and  $20.0 \pm 3.3 \mu\text{g/g}$  after stress, showing no significant

change after stress.

The HA contents in the 2-week of the obstruction group were  $31.6 \pm 8.0 \mu\text{g/g}$  in the pre-stress level, and  $33.5 \pm 9.2 \mu\text{g/g}$  after stress, in the 3-week group  $33.8 \pm 6.2 \mu\text{g/g}$  in the pre-stress level, and  $26.2 \pm 4.7 \mu\text{g/g}$  after stress, and in the 4-week group  $40.4 \pm 9.8 \mu\text{g/g}$  in the pre-stress level, and  $30.6 \pm 4.9 \mu\text{g/g}$  after stress. Thus, in the 3,4-week of the obstruction group, the HA contents after stress showed significantly lower levels as compared with the pre-stress levels ( $p < 0.05$ , Fig. 1).

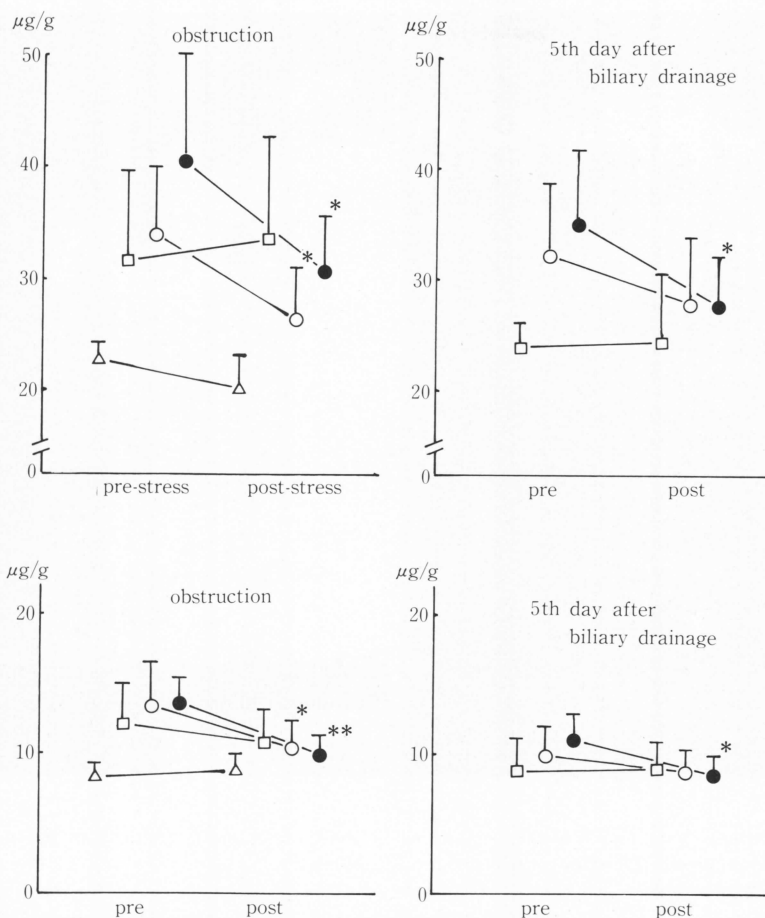
The HA contents in the 2-week of the drainage group were  $23.9 \pm 2.2 \mu\text{g/g}$  in the pre-stress level, and  $24.3 \pm 6.1 \mu\text{g/g}$  after stress, in the 3-week group  $32.1 \pm 6.5 \mu\text{g/g}$  in the pre-stress level, and  $27.7 \pm 6.2 \mu\text{g/g}$  after stress, and in the 4-week group  $35.0 \pm 6.7 \mu\text{g/g}$  in the pre-stress level, and  $27.6 \pm 4.6 \mu\text{g/g}$  after stress. Thus, in the 4-week of the drainage group, the HA content after stress showed significantly lower level as compared with the pre-stress level ( $p < 0.05$ , Fig. 1).

### 3) Changes in the 5-HT contents in the gastric mucosa

The 5-HT contents in the control group were  $8.3 \pm 1.0 \mu\text{g/g}$  in the pre-stress level, and  $8.7 \pm 1.3 \mu\text{g/g}$  after stress, showing no significant change after stress.

The 5-HT contents in the 2-week of the obstruction group were  $12.1 \pm 2.9 \mu\text{g/g}$  in the pre-stress level, and  $10.7 \pm 2.5 \mu\text{g/g}$  after stress, in the 3-week group  $13.5 \pm 3.0 \mu\text{g/g}$  in the pre-stress level, and  $10.3 \pm 2.1 \mu\text{g/g}$  after stress, and in the 4-week group  $13.6 \pm 1.9 \mu\text{g/g}$  in the pre-stress level, and  $9.8 \pm 1.5 \mu\text{g/g}$  after stress. Thus, in the 3,4-week of the obstruction group, the 5-HT contents after stress showed significantly lower levels as compared with the pre-stress levels ( $p < 0.05$ , Fig. 1).

The 5-HT contents in the 2-week of the drainage group were  $8.7 \pm 2.5 \mu\text{g/g}$  in the pre-stress level, and  $8.8 \pm 2.1 \mu\text{g/g}$  after stress, in the 3-week group  $9.7 \pm 2.3 \mu\text{g/g}$  in the pre-stress level, and  $8.6 \pm 1.8 \mu\text{g/g}$  after stress, and in the 4-week group  $10.9 \pm 2.0 \mu\text{g/g}$  in the pre-stress level, and  $8.5 \pm 1.3 \mu\text{g/g}$  after stress. Thus, in the 4-week of the drainage group, the 5-HT content after stress showed significantly lower level as compared with the pre-stress level ( $p < 0.05$ , Fig. 1).



**Fig. 1.** Changes in histamine (top) and serotonin (bottom) contents in the gastric mucosa on cold restraint for 30 min

\*Significance of the difference between means of the pre- and post-restraint value within each group.  $\Delta$ — $\Delta$  control,  $\square$ — $\square$  2 weeks,  $\circ$ — $\circ$  3 weeks,  $\bullet$ — $\bullet$  4 weeks ( $M \pm SD$ ,  $n = 8-9$ , \* $p < 0.05$ , \*\* $p < 0.01$ )

4) Findings in the microvascular structure in the gastric mucosa and the distribution of erythrocytes within capillary systems in the gastric mucosa

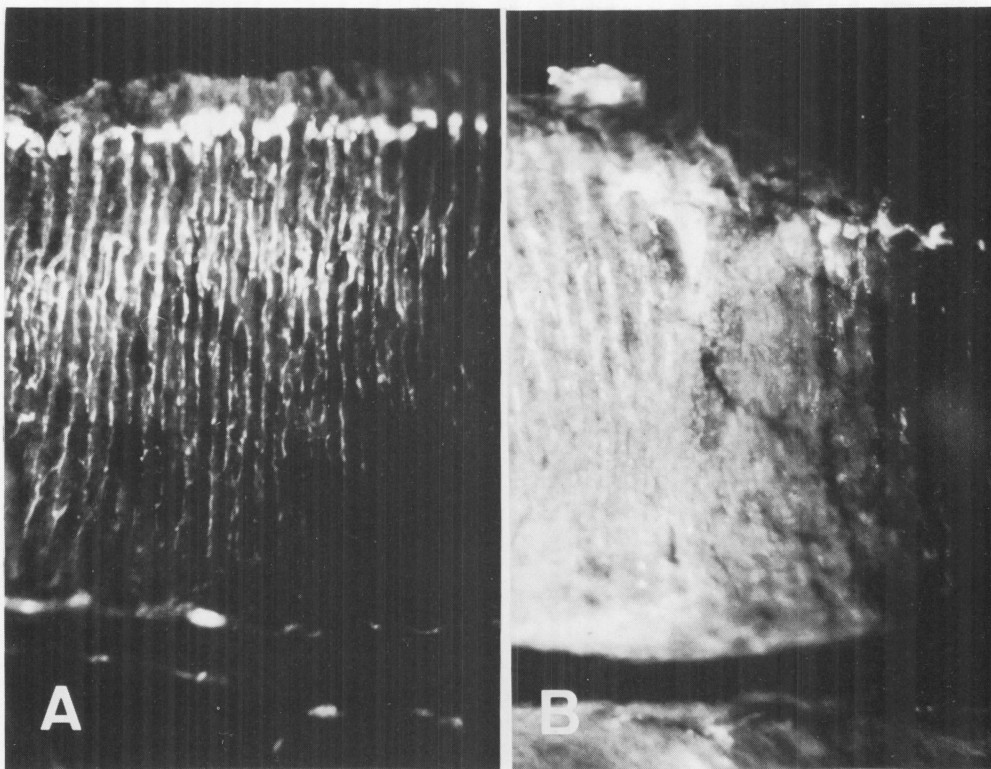
In the control group after stress, a mild distension of venous capillaries was observed, as compared before stress, but FITC-dextran fluorescence in capillaries and venous capillaries was noted rather successfully (Fig. 2A), whereas in the 4-week of the obstruction group after stress, FITC-dextran fluorescence in capillary systems was markedly weakened (Fig. 2B).

In the control group, erythrocytes existed very few in capillary systems after stress (Fig. 3A), whereas it was observed that numerous erythro-

cytes existed in capillary systems in the 4-week of the obstruction group after stress, which indicated congestion (Fig. 3B).

## DISCUSSION

As a result of giving load of cold restraint for 30 min on rats in the control, the histamine and serotonin contents in the gastric mucosa showed no significant change afterwards, and gastric mucosal microcirculation was prevented successfully. Furthermore, the incidence of AGML was kept low. Accordingly, cold restraint for 30 min used by the authors for the animals in the control was considered to be a very mild amount of stress.



**Fig. 2.** Microangiograph in the gastric mucosa in the control group (A) and in the 4-week of the obstruction group (B) after cold restraint for 30 min

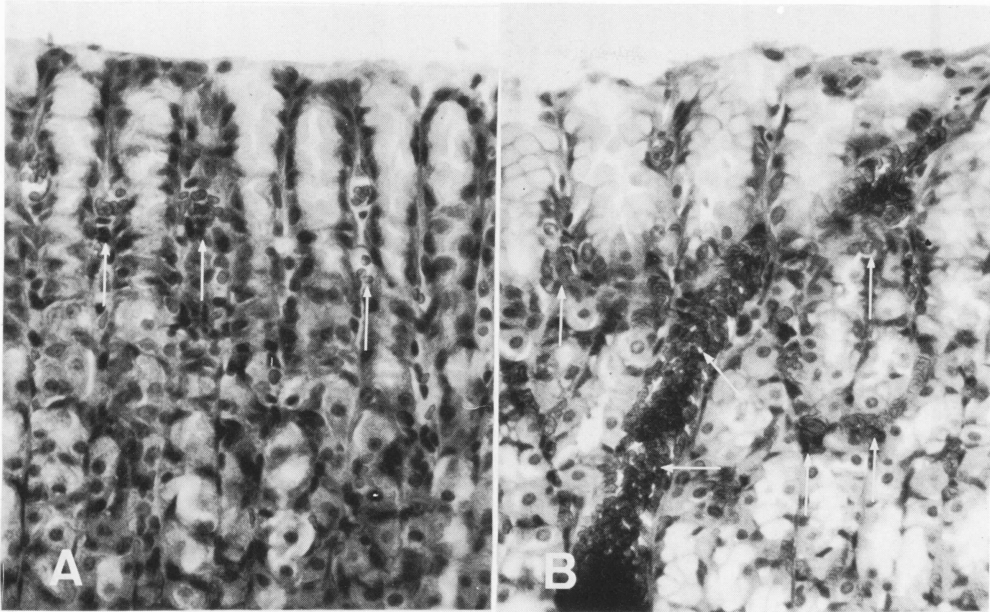
A: The capillary systems are clearly visualized.  $\times 100$

B: The weakening of FITC fluorescence in capillaries and venous capillaries are observed.  $\times 100$

On the other hand, when a stress considered to be mild was given on rats with obstructive jaundice and those on which biliary drainage was given, both amine contents in the 3.4-week of the obstruction group and in the 4-week of the drainage group were markedly decreased. This result can be considered due to the acceleration of the release of both amines in the gastric mucosa after stress. In accordance with the periods when both amines showed significant decrease, the incidence of AGML was markedly increased. Furthermore, when microcirculation in the gastric mucosa was observed in these periods, disturbance of gastric mucosal microcirculation due to congestion seemed to be induced. It is considered therefore that in the gastric mucosa in the 3.4-week group of rats with obstructive jaundice, acceleration of the release of both amines is easily induced due to a mild stress, and in addition, disturbance of gastric

mucosal microcirculation due to congestion is induced<sup>6,7)</sup>, and a preparatory state for AGML, i.e. a state in which AGML may occur at any time, will be formed. While such a pathological state can be improved in the 3-week group of rats with obstructive jaundice by biliary drainage, in the 4-week group it may not be improved any more.

In these periods, the storage of both amines in the gastric mucosa was increased<sup>6,7)</sup>. It can be considered therefore when the duration of obstructive jaundice is prolonged and the storage of both amines is increased, so that it is considered that the increase in the storage of both amines may be associated with the formation of the preparatory state for AGML.



**Fig. 3.** Microscopic findings of the gastric mucosa in the control group (A) and in the 4-week of the obstruction group (B) after cold restraint for 30 min

A: Very few erythrocytes (↑) can be seen in capillaries and venous capillaries. H-E stain, × 200

B: Numerous erythrocytes (↑) can be seen in capillaries, venous capillaries and collecting venule. H-E stain, × 200

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